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PRINCIPLE INVESTIGATOR: Hye-Sook Seo, Ph.D.

CONTRACTING ORGANIZATION: The University of Texas
M.D. Anderson Cancer Center
Houston, TX 77030

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14. ABSTRACT

My goal of the study is to know whether LGD1069 and LG100268 (rexinoids) suppress the growth of breast cancer cells, and how these drugs act to inhibit breast cancer development. For that purpose, I tested 5 different breast cell lines; 1 human mammary epithelial cell line (HMEC), 2 different ER-positive cell lines (MCF-7 and T47D) and 2 different ER-negative breast cancer cell lines (MDA-MB-231 and MDA-MB-435). By MTS assay, I found that both LGD1069 and LG100268 inhibited significantly normal HMEC cell growth at 10 uM. I also found that LGD1069 strongly suppressed the growth of T47D (ER-positive) by dose-dependent manner. LGD1069 also induced a mild inhibition of MDA-MB-231 (ER-negative) at 10 uM. MCF-7 and MDA-MB-435 did not have growth suppression by LGD1069 at 10 uM. LG100268 did affect little the cell growth in all 4 breast cancer cell lines suggesting its weak activity compare to LGD1069. To investigate the mechanism by which LGD1069 suppresses breast tumorigenesis, we have studied the genes modulated by LGD1069. We treated 2 different cell lines (T47D and MDA-MB-231) with LGD1069 at 10 uM for 12 hrs, extracted RNA and then submitted to Affymetrix Microarray. According to the data, we found several interesting genes induced by LGD1069. We will study their functions. In this report, I present genes regulated by LGD1069 in MDA-MB-231, ER-negative cell line.

15. SUBJECT TERMS

retinoid, rexinoid, HMEC, T47D, MDA-MB-231, LGD1069, LG100268, breast cancer

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INTRODUCTION

Molecular mechanisms by which retinoids prevent breast cancer development

The ultimate goal of the fundamental and clinical investigators for breast cancer is to develop new and effective drug for the prevention and/or treatment of breast cancer with minimal toxicity. For the moment, tamoxifen and raloxifen, selective estrogen receptor modulators (SERM) are widely used to prevent and treat breast cancer. However, they suppress pre-dominently the development of estrogen receptor (ER)-positive breast cancer and not ER-negative breast cancer. ER-negative breast cancers are more aggressive and poor in the prognosis. These facts point out the urgent need for strategie to prevent ER-negative breast cancer. Potential candidates for the prevention and treatment of ER-negative breast cancer are aromatase inhibitors, COX inhibitors, EGFR inhibitors and retinoids. Among them, retinoids have been demonstrated to be the most effective agents at suppressing ER-negative mammary tumorigenesis in preclinical models (Lu et al., 2003; Wu et al., 2000; Wu et al., 2002a; Wu et al., 2002b; Wu et al., 2006).

Under this concept, we evaluated the efficacy of the retinoids on the growth of breast cells. For this task, we especially used the rexinoids, RXR-binding ligands (rexinoids) which are promising compounds for the chemoprevention and treatment of the breast cancer.

Rexinoids are important in controlling apoptosis and can function in a ligand-dependent or ligand-independent manner (Nagy et al., 1998; Lippman and Lotan, 2000). Importantly, rexinoids are known to suppress both ER-positive and ER-negative mammary tumor development with reduced toxicity compared to RAR-selective retinoids (Gottardis et al., 1996; Wu et al., 2002a, 2002b). Rexinoids are also active in animals in tamoxifen-resistant breast cancer (Bischoff et al., 1999; Lippman and Lotan, 2000) and in ATRA-resistant breast cancer cells (Crowe and Chandraratna, 2004). Hence, rexinoids seems to be more efficient and promising chemopreventive and therapeutic drugs compare to RAR-selective ligands. Among them, LGD1069 (Bexarotene) was confirmed as safe and well tolerated agent in clinical trials of cutaneous T-cell lymphoma, beast cancer and lung cancer (Farol and Hymes, 2004; Rigas and Dragnev, 2005).

My goal of the study is to know whether LGD1069 and LG100268 (rexinoids) suppress the growth of breast cancer cells, and how these drugs act to inhibit breast cancer development. For that purpose, I tested 5 different breast cell lines; 1 human mammary epithelial cell line (HMEC), 2 different ER-positive cell lines (MCF-7 and T47D) and 2 different ER-negative breast cancer cell lines (MDA-MB-231 and MDA-MB-435). By MTS assay, I found that both LGD1069

and LG100268 inhibited significantly normal HMEC cell growth at 10 uM. I also found that LGD1069 strongly suppressed the growth of T47D (ER-positive) by dose-dependent manner. LGD1069 also induced a mild inhibition of MDA-MB-231 (ER-negative) at 10 uM. MCF-7 and MDA-MB-435 did not have growth suppression by LGD1069 at 10 uM. LG100268 did affect little the cell growth in all 4 breast cancer cell lines suggesting its weak activity compare to LGD1069. To investigate the mechanism by which LGD1069 suppresses breast tumorigenesis, we have studied the genes modulated by LGD1069. We treated 2 different cell lines (T47D and MDA-MB-231) with LGD1069 at 10 uM for 12 hrs, extracted RNA and then submitted to Affymetrix Microarray. According to the data, we found several interesting genes induced by LGD1069. We will study their functions. In this report, I present genes regulated by LGD1069 in MDA-MB-231, ER-negative cell line.

BODY

Molecular mechanisms by which retinoids prevent breast cancer development

For my first year grant research (2004-2005), our sub-title of the study was "Identification of RXR alpha target genes that are involved in the suppression of the growth of human mammary epithelial cells (HMEC)". For this study, we evaluated the growth suppressive activity of RXRα compound, Ro25-7328 on HMEC, MCF-7 and T47D. We found that Ro 25-7328 suppressed the growth of all three cell lines, HMEC, MCF-7 and T47D. We especially targeted the HMEC and MCF-7 cell lines, treated with Ro25-7328, extracted total RNA and submitted to the Affymetrix microarray to identify RXRα target genes. We found several interesting genes from Affymetrix microarray data of these two cell lines. We selected some genes, confirmed their expression levels by quantitative real-time RT-PCR and western blot. The study of their functions was subjected to this second year research. Thus, we started to work on that, this 2nd year.

According to my statement of work, we needed to use LGD1069 and LG100268 compounds (rexinoids) which randomly bind to RXR isoforms and to work on LGD1069-target gene IGFBP-6 to investigate the role of this gene in breast cancer development. However, I could not obtain these compounds in the first of my year due to my transition (from Baylor College of Medicine, Houston, TX to UT University MD Anderson Cancer Center, Houston, TX) and the administrative process between our institution and Ligand Pharmaceuticals. That is why I worked on another specific rexinoid, Ro25-7328 which exclusively bind RXR α isoform. It was very meaningful work since RXR α seems to play a critical role in tumor suppression.

Fortunately, I received this year LGD1069 and LG100268 from Ligand Pharmaceuticals, and started to test the activity and function of these compounds. First we examined the efficacy of the rexinoids on the growth of breast cell lines; normal breast cells (HMEC), ER-positive breast cancer cells(MCF-7 and T47D), ER-negative breast cancer cells (MDA-MB-231 and MDA-MB-435). For the next step, we performed Affymetrix microarray to identify the genes that involved in the growth suppression induced by LGD1069 and LG100268. So this year, our study is composed of two parts: 1. study of Ro25-7328, 2. study of LGD1069 and LG100268.

I. Ro25-7328 (RXRα-specific ligand)

1. RXR α target genes of HMEC by Affymetrix microarray – the function of the E-cadherin on the growth suppression of HMEC induced by Ro25-7328

First year, we identified genes regulated by RXRα–specific ligand, Ro25-7328 in normal breast cells, HMEC. RXRα compound, Ro25-7328 induced strong growth suppression in HMEC. RXRα-regulated genes include integrin beta 4, integrin alpha 6, cadherin 1, type 1, E-cadherin (epithelial) (CDH1), paxillin (PAX), BCL2-associated X protein (BAX), forkhead box O3A (FOXO3A), signal transducer and activator of transcription 3 (STAT3), collagen, type VI, alpha 3, cell division cycle 42 (CDC42). We also confirmed their expression levels by quantitative real-time RT-PCR assays and western blot analysis.

This second year, we selected cadherin 1, type 1, E-cadherin (epithelial) (CDH1), and started to study the function of this gene in HMEC. Our objective was whether CDH1 plays an important role in the growth suppressive activity of Ro25-7328 in HMEC. Our ongoing experiments are composed of siRNA transfection, exogeneous addition of recombinant human CDH1 in the cells.

Exp. No.	Subject of Exp.	Process	Materials
Exp.1-HMEC	Transfection of siRNA for CDH1 in HMEC	ongoing	Obtained E-cadherin ON-TARGET plus SMARTpool from Dharmacon
Exp.2-HMEC	Exogeneous addition of recombinant human CDH1 in HMEC and MTS assay	ongoing	Obtained recombinant human E-cadherin/Fc chimera from R&D systems
Exp.3-HMEC	Determine the promoter activity of CDH1 using luciferase assay	scheduled	Luciferase reporter gene constructs containing wild-type human E-cadherin promoter sequences would be generated or provided from other Lab.

After accomplishing the Exp. described above, we could comprehend more concretely the role of E-cadherin in the growth suppression of HMEC induced by Ro25-7328. We will also investigate other RXRα-regulated genes.

2. RXR α target genes of MCF-7 by Affymetrix microarray – the function of the TGF beta 1 and 2 on the growth suppression of MCF-7 induced by Ro25-7328

We also identified genes regulated by RXR α in MCF-7, retinoid-sensitive cell line. RXR α compound, Ro25-7328 induced mild growth suppression in this cell line. In MCF-7, we found several interesting genes which could be involved in cell growth inhibition induced by Ro25-7328. They include transforming growth factor, beta 2, protein kinase C, delta binding protein, cathepsin S, transforming growth factor, beta 1 (Camurati-Engelmann disease), basigin, myeloid cell leukemia sequence 1 (BCL2-related) (MCL-1), BCL2-like 1(BCL2L1). In this second year, we selected especially TGFβ1, confirmed its expression level and worked its function in MCF-7. We found that the mRNA level of TGFβ1 was not affected in Ro25-7328–treated cells compared to DMSO-treated cells. However, we found the decrease of TGFβ1 protein level in treated MCF-7 cells (Figure 1).

We then explored whether TGFβ1 plays an important role in the growth suppressive activity of Ro25-7328 in MCF-7. Our ongoing experiments are composed of exogeneous addition of recombinant human TGFβ1 as well as neutralizing TGFβ1 antibody in the cells. We also work on TGFβ2 at the same time.

Exp. No.	Subject of Exp.	Process	Materials
Exp.1-MCF-7	Confirmation of the expression level of TGFβ2 in MCF-7	ongoing	Obtained primer set and antibody Superarray and R&D system
Exp.2-MCF-7	Exogeneous addition of recombinant human TGFβ1 and TGFβ2 in MCF-7 and MTS assay	ongoing	Obtained recombinant human TGFβ1 and TGFβ2 from R&D systems
Exp.3-MCF-7	Neutralization of TGFβ1 and TGFβ2 bioactivity	ongoing	Obtained anti-TGFβ1 and anti-TGFβ2 from R&D systems

After accomplishing the Exp. described above, we could comprehend more concretely the role of TGFβ signaling in the growth suppression of MCF-7 induced by Ro25-7328. We will also investigate other RXRα-regulated genes.

II. LGD1069 and LG100268 (rexinoid)

1. Effect of LGD1069 and LG100268 on the growth of normal breast cells, HMEC

The molecular structure of LGD1069 and LG100268 is shown in Figure 2. We first evaluated the effect of our rexinoid, LGD1069 and LG100268 on the growth of HMEC. For that purpose, we treated cells with LGD1069 and/or LG100268 at the concentrations ranging from 10 nM to 10 μM, then measured relative cell growth rate by MTS assay. We found that both LGD1069 and LG100268 suppressed cell growth rate of HMEC by dose-dependent manner (Figure 3). After 8 days of treatment, we found that the growth of HMEC was strongly inhibited at the dose of 10 μM. On the other hand, when cells were submitted to the co-treatment of LGD1069 and LG100268, we could not observe the synergistic effect of the ligands, LGD1069 and LG100268.

2. Effect of LGD1069 and LG100268 on the growth of ER-positive breast cancer cells, MCF-7 and T47D

We also measured the cell growth rate of MCF-7 and T47D, after their treatment with LGD1069 and LG100268 (Figure 4). Surprisingly, both LGD1069 and LG100268 did not affect the cell growth rate of MCF-7 even at the strongest dose, 10 μM. However, T47D displayed its sensitivity to LGD1069 and LG100268. The rexinoids strongly suppressed the cell growth of T47D by dose-dependent manner after 10 days of treatment. Especially, it seems that LGD1069 has stronger activity compare to LG100268 for the cell growth of T47D.

3. Effect of LGD1069 and LG100268 on the growth of normal breast cells, HMEC

We studied whether LGD1069 and LG100268 influence the cell growth of MDA-MB-231 and MDA-MB-435. Surprisingly, we found that rexinoid, especially MDA-MB-231 demonstrated its sensitivity to rexinoid while MDA-MB-435 was insensitive. Rexinoid, especially LGD1069 induced a mild inhibition (20 % inhibition) of the cell growth of MDA-MB-231 at the dose of 10 μM (Figure 5). This result indicates that LGD1069 can inhibit the growth of ER-negative breast cancer with therapeutic potency.

4. Determine the regulated genes of MDA-MB-231 induced by LGD1069 and LG100268

Since rexinoid induced a mild inhibition of cell growth of MDA-MB-231, we investigated the genes regulated by LGD1069 and LG100268 to know which signaling pathway is involved in rexinoid-induced cell growth inhibition. Gene expression profiles were established by using Affymetrix microarray (human genome U133A 2.0).

For that purpose, we treated MDA-MB-231 cells with LGD1069 and LG100268 using the concentration which most strongly suppressed the cell growth (10 μM), and total RNA sample was harvested after 12h; this time point was selected for study since retinoid treatment would likely regulate the expression of genes earlier than 24h (Ma et al., 2003). We then examined changes in gene expression by using the microarray to investigate which genes are related to cell growth inhibition induced by the rexinoid. This may lead us to find a novel molecule which can suppress the development of ER-negative breast cancer.

A. LGD1069-regulated genes

In MDA-MB-231, we identified 333 genes up-regulated and 319 genes down-regulated by LGD1069 with changes in fold induction greater than 2 fold (Table 1 and 2). Among them, we found several hypothetical proteins, CDC14 cell division cycle 14 homolog A (S. cerevisiae), recombination activating gene 2, tumor protein D52, MDM2, ITGA4, ADh1B, NF2 and cathepsin S. We selected our major genes of interest by referring to PathArt program which demonstrate the relationship between genes by several signaling pathways (Figure 6-11).

B. LG100268-regulated genes

In MDA-MB-231, we identified 116 genes up-regulated and 431 genes down-regulated by LG100268 with changes in fold induction greater than 2 fold (Table 3 and 4). Among them, we found several hypothetical proteins, Zinc finger protein 423, cyclin-dependent kinase inhibitor 1C (p57, Kip2), eukaryotic translation initiation factor 5A, Transcription factor 4, MDM2, FGF2, GNRH1 and annexin A9. We selected our major genes of interest by referring to PathArt program which demonstrate the relationship between genes by several signaling pathways (Figure 12-14).

We will select genes from them and confirm the expression level by quantitative real-time RT-PCR and western blot analysis. We will study the function of those genes to know whether they play important role in the suppression of breast cancer development induced by LGD1069 and LG100268.

We are also in the process of Affymetrix microarray for other cell lines, HMEC and T47D that have strong sensitivity for LGD1069 and LG100268.

Exp. No.	Subject of Exp.	Process	Materials
Exp.1-array	Gene expression profile in HMEC treated with LGD1069 and LG100268	ongoing	Affymetrix microarray (human genome U133A 2.0).
Exp.2-array	Gene expression profile in T47D treated with LGD1069 and LG100268	ongoing	Affymetrix microarray (human genome U133A 2.0).

KEY RESEARCH ACCOMPLISHMENTS

1. LGD1069 and LG100268 strongly suppressed the growth of normal breast cells displaying their potential chemopreventive activity.

2. LGD1069 strongly suppressed the growth of T47D cells displaying its therapeutic role in ER-positive breast cancer.

3. LGD1069 strongly suppressed the growth of MDA-MB-231 cells displaying its therapeutic role in ER-negativee breast cancer.

4. Gene profiling for rexinoid-target genes in MDA-MB-231.

REPORTABLE OUTCOMES

We have intention to publish our data in peer-reviewed papers and present in annual breast cancer meeting.

CONCLUSIONS

In the second year, we tested the efficacy of rexinoid (LGD1069 and LG100268) on the growth of breast cells. Rexinoid is effective for the prevention and treatment for the breast cancer. We found that LGD1069 and LG100268 strongly suppressed the growth of HMEC at 10 μM. We also found that T47D was very sensitive to LGD1069 and LG100268 showing dose-dependent cell growth inhibition. However, another ER-positive cell line, MCF-7 was insensitive to rexinoid; both LGD1069 and LG100268 failed to suppress cell growth of MCF-7. This indicates that rexinoid should be selectively used even within ER-positive breast cancer.

Remarkably, we found that LGD1069 induced a mild inhibition of ER-negative breast cancer cell line, MDA-MB-231 (20 %) indicating its potential therapeutic role for the ER-negative breast cancer. Thus, we performed gene profiling using Affymetrix microarray, to identify rexinoid-regulated genes in MDA-MB-231.

In MDA-MB-231, we identified 333 genes up-regulated and 319 genes down-regulated by LGD1069 with changes in fold induction greater than 2 fold. Among them, we found several hypothetical proteins, CDC14 cell division cycle 14 homolog A (S. cerevisiae), recombination activating gene 2, tumor protein D52 and cathepsin S.

We also identified, 116 genes up-regulated and 431 genes down-regulated by LG100268 in MDA-MB-231, with changes in fold induction greater than 2 fold. Among them, we found several hypothetical proteins, Zinc finger protein 423, cyclin-dependent kinase inhibitor 1C (p57, Kip2), eukaryotic translation initiation factor 5A, Transcription factor 4 and annexin A9.

We will select the genes which are implicated in growth inhibition of breast cells induced by Ro25-7328. Profound investigation of those may lead us to clarify how rexinoidt functions to inhibit breast cell growth.

This study could help us to find new preventive/therapeutic target for breast cancer, and may contribute to develop novel molecule which could inhibit breast cancer development.

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Decrease of TGFβ1 protein level induced by Ro25-7328 in MCF-7

Gene name	*Fold change*
transforming growth factor, beta 1 (Camurati-Engelmann disease)	-3.34

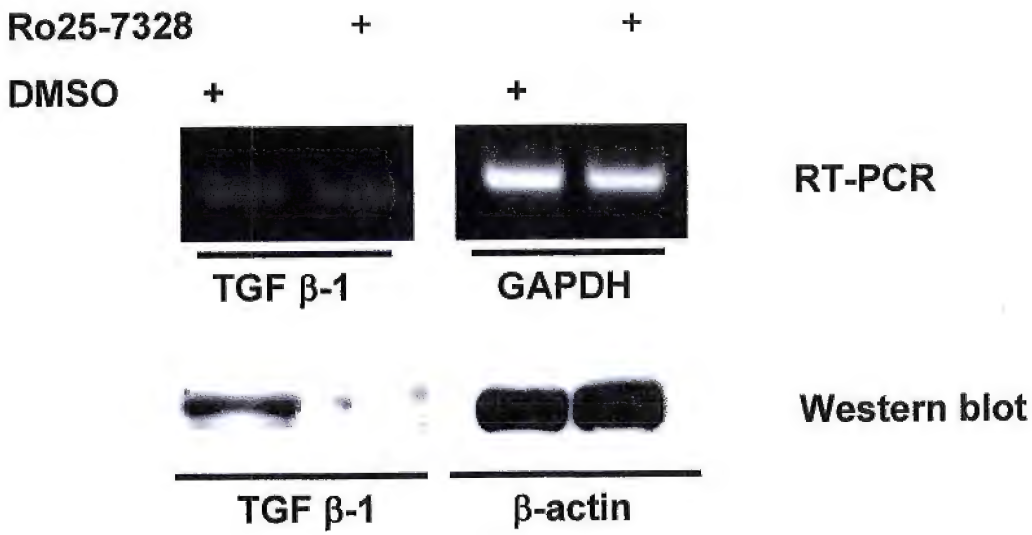
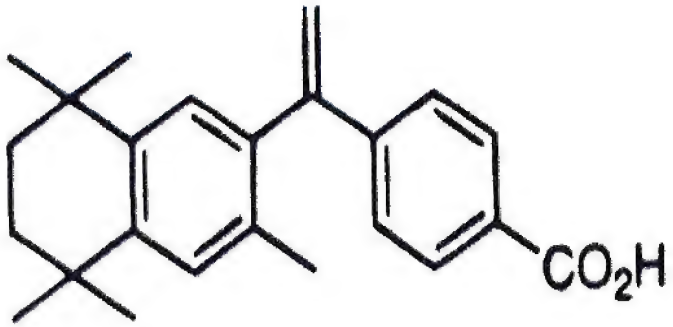
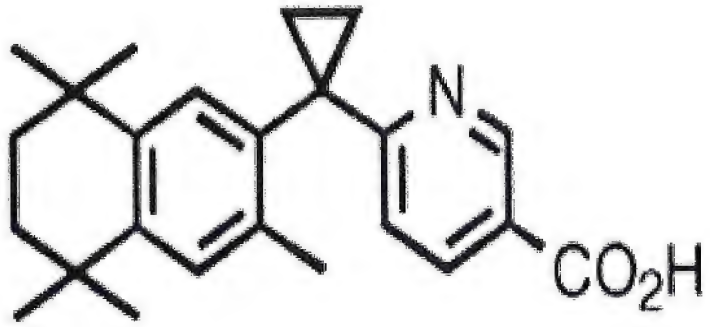


Figure 1

Molecular structure of LGD1069 and LG100268



LGD1069



LG100268

Figure 2

The effect of LGD1069 and LG100268 on the growth of HMEC

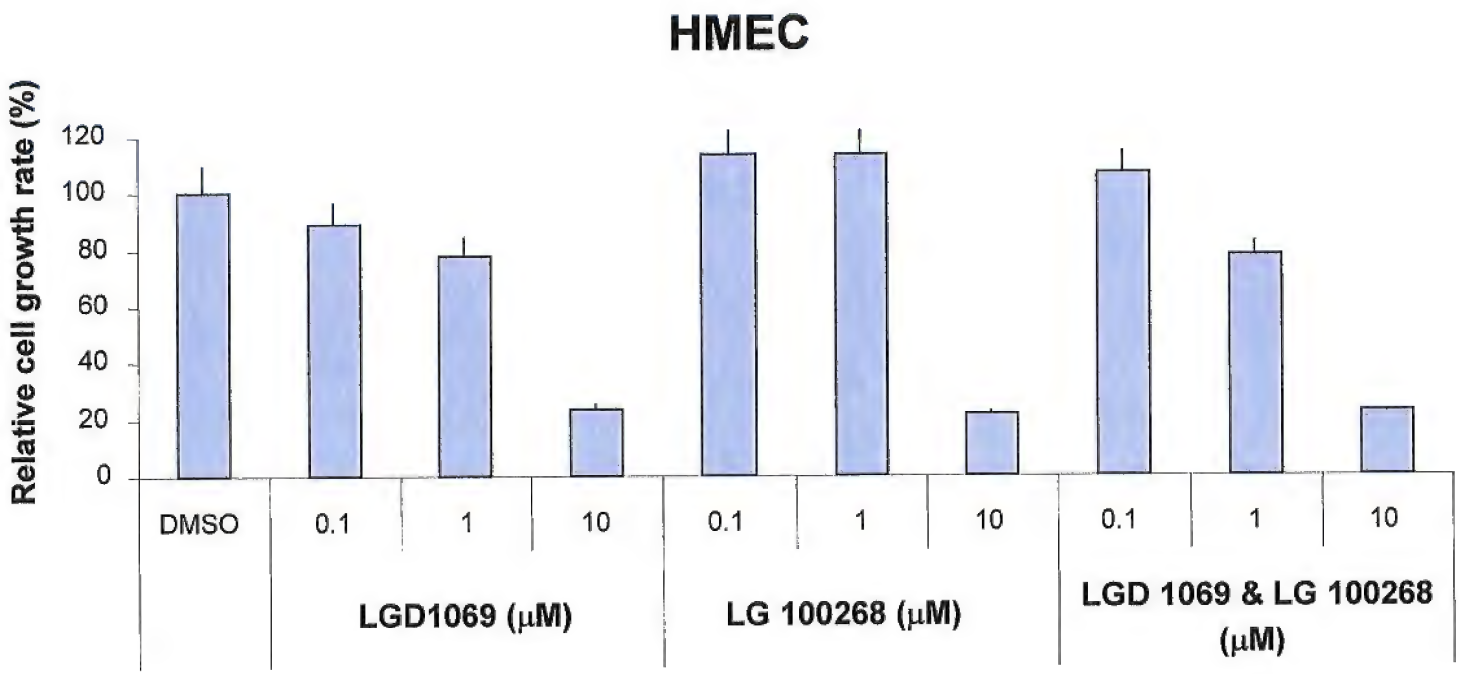


Figure 3

The effect of LGD1069 and LG100268 on the growth of MCF-7 and T47D

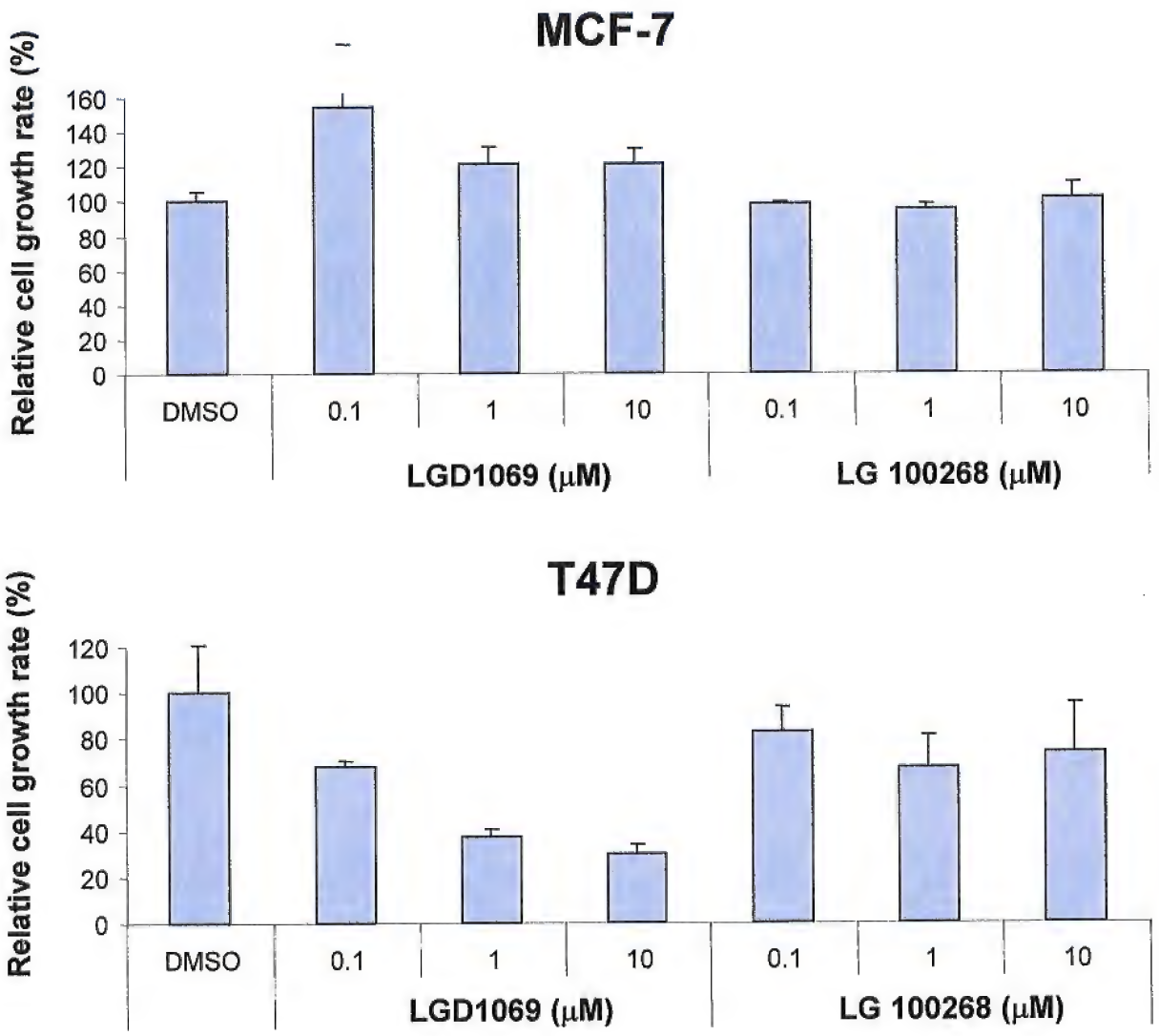


Figure 4

The effect of LGD1069 and LG100268 on the growth of MDA-MB-231 and MDA-MB-435

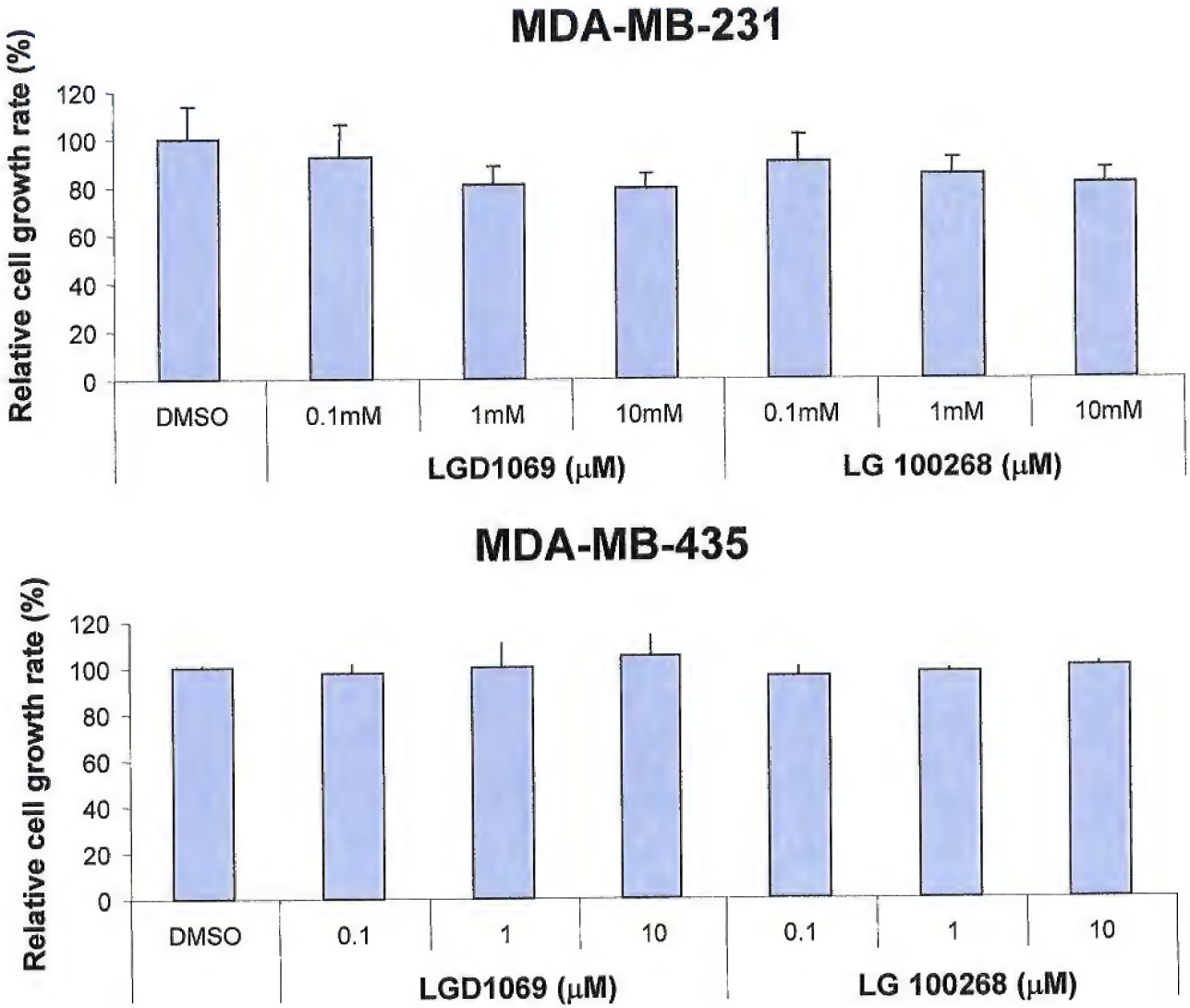


Figure 5

Table 1. Genes Up-regulated by LGD1069 in MDA-MB-231

probe set	gene	fold change
219948_x_at	hypothetical protein FLJ21934	232.43
209672_s_at	hypothetical protein FLJ20323	69.61
207750_at	gb:NM_018510.1 /DEF=Homo sapiens hypothetical protein PRO1866 (PRO1866), mRNA. /FEA=mRNA /GEN=PRO1866 /PROD=hypothetical protein PRO1866 /DB_XREF=gi:8924091 /UG=Hs.283031 hypothetical protein PRO1866 /FL=gb:AF119858.1 gb:NM_018510.1	30.5
203603_s_at	zinc finger homeobox 1b	10.18
217698_at	Consensus includes gb:AV651668 /FEA=EST /DB_XREF=gi:9872682 /DB_XREF=est:AV651668 /CLONE=GLCCSC04 /UG=Hs.282480 ESTs	10.11
AFFX-r2-Ec-bioB-M_at	E. coli /GEN=bioB /DB_XREF=gb:J04423.1 /NOTE=SIF corresponding to nucleotides 2393-2682 of gb:J04423.1 /DEF=E.coli 7,8-diamino-pelargonic acid (bioA), biotin synthetase (bioB), 7-keto-8-amino-pelargonic acid synthetase (bioF), bioC protein, and dethiobiot	9.76
205386_s_at	Mdm2, transformed 3T3 cell double minute 2, p53 binding protein (mouse)	9.65
216119_s_at	chromosome 20 open reading frame 28	9.42
AFFX-BioB-M_at	E. coli /GEN=bioB /DB_XREF=gb:J04423.1 /NOTE=SIF corresponding to nucleotides 2482-2739 of gb:J04423.1 /DEF=E.coli 7,8-diamino-pelargonic acid (bioA), biotin synthetase (bioB), 7-keto-8-amino-pelargonic acid synthetase (bioF), bioC protein, and dethiobiot	9.32
209613_s_at	alcohol dehydrogenase IB (class I), beta polypeptide	8.85
AFFX-r2-Ec-bioB-3_at	E. coli /GEN=bioB /DB_XREF=gb:J04423.1 /NOTE=SIF corresponding to nucleotides 2772-3004 of gb:J04423.1 /DEF=E.coli 7,8-diamino-pelargonic acid (bioA), biotin synthetase (bioB), 7-keto-8-amino-pelargonic acid synthetase (bioF), bioC protein, and dethiobiot	8.78
217194_at	Consensus includes gb:AB007970.1 /DEF=Homo sapiens mRNA, chromosome 1 specific transcript KIAA0501. /FEA=mRNA /DB_XREF=gi:3413945 /UG=Hs.223020 Homo sapiens mRNA, chromosome 1 specific transcript KIAA0501	7.08
205524_s_at	hyaluronan and proteoglycan link protein 1	7.06
215514_at	Consensus includes gb:AL080072.1 /DEF=Homo sapiens mRNA; cDNA DKFZp564M0616 (from clone DKFZp564M0616). /FEA=mRNA /DB_XREF=gi:5262482 /UG=Hs.21195 Homo sapiens mRNA; cDNA DKFZp564M0616 (from clone DKFZp564M0616)	6.85
214774_x_at	trinucleotide repeat containing 9	6.7
215526_at	Consensus includes gb:AL050145.1 /DEF=Homo sapiens mRNA; cDNA DKFZp586C2020 (from clone DKFZp586C2020). /FEA=mRNA /DB_XREF=gi:4884356 /UG=Hs.225986 Homo sapiens mRNA; cDNA DKFZp586C2020 (from clone DKFZp586C2020)	6.22
211091_s_at	neurofibromin 2 (bilateral acoustic neuroma)	6.21

221959_at	hypothetical protein MGC39325	6.11
206863_x_at	gb:U76376.1 /DB_XREF=gi:1923234 /GEN=HRK /FEA=FLmRNA /CNT=9 /TID=Hs.87247.0 /TIER=ConsEnd /STK=0 /UG=Hs.87247 /LL=8739 /DEF=Homo sapiens activator of apoptosis Hrk (HRK) mRNA, complete cds. /PROD=activator of apoptosis Hrk /FL=gb:NM_003806.1 gb:U76376.1	6.09
206202_at	mesenchyme homeo box 2 (growth arrest-specific homeo box)	5.75
205288_at	CDC14 cell division cycle 14 homolog A (S. cerevisiae)	5.62
220931_at	hypothetical protein MGC5590	5.4
216795_at	CDNA: FLJ23194 fis, clone REC00490	5.29
206410_at	nuclear receptor subfamily 0, group B, member 2	5.23
207647_at	chromodomain protein, Y-linked, 1 /// chromodomain protein, Y-linked, 1B	5.19
215112_x_at	MCF.2 cell line derived transforming sequence-like 2	5.11
216775_at	ubiquitin specific protease 53	4.9
220109_at	transferrin	4.88
217132_at	Clone 24587 mRNA sequence	4.86
216737_at	CDNA: FLJ20872 fis, clone ADKA02604	4.84
220036_s_at	lipocalin-interacting membrane receptor	4.7
AFFX-r2-Ec-bioD-3_at	E. coli /GEN=bioD /DB_XREF=gb:J04423.1 /NOTE=SIF corresponding to nucleotides 5312-5559 of gb:J04423.1, not 100% identical /DEF=E.coli 7,8-diamino-pelargonic acid (bioA), biotin synthetase (bioB), 7-keto-8-amino-pelargonic acid synthetase (bioF), bioC pro	4.66
220564_at	chromosome 10 open reading frame 59	4.64
211611_s_at	tenascin XB /// tenascin XB /// cAMP responsive element binding protein-like 1 /// cAMP responsive element binding protein-like 1	4.61
AFFX-BioDn-3_at	E. coli /GEN=bioD /DB_XREF=gb:J04423.1 /NOTE=SIF corresponding to nucleotides 5286-5570 of gb:J04423.1, not 100% identical /DEF=E.coli 7,8-diamino-pelargonic acid (bioA), biotin synthetase (bioB), 7-keto-8-amino-pelargonic acid synthetase (bioF), bioC pro	4.49
207272_at	zinc finger protein 80 (pT17)	4.49
210690_at	killer cell lectin-like receptor subfamily C, member 4	4.47
216625_at	Consensus includes gb:AL050032.1 /DEF=Homo sapiens mRNA; cDNA DKFZp566F1224 (from clone DKFZp566F1224). /FEA=mRNA /DB_XREF=gi:4884272 /UG=Hs.306307 Homo sapiens mRNA; cDNA DKFZp566F1224 (from clone DKFZp566F1224)	4.37
207245_at	UDP glycosyltransferase 2 family, polypeptide B17	4.35
208014_x_at	neuronal thread protein AD7c-NTP	4.32
214767_s_at	heat shock protein, alpha-crystallin-related, B6	4.31
216697_at	Triple functional domain (PTPRF interacting)	4.28
222341_x_at	Consensus includes gb:AW973235 /FEA=EST /DB_XREF=gi:8163081 /DB_XREF=est:EST385333 /UG=Hs.293697 ESTs	4.27
207262_at	apolipoprotein F	4.25
222320_at	Consensus includes gb:AW970584 /FEA=EST /DB_XREF=gi:8160429 /DB_XREF=est:EST382665 /UG=Hs.291033 ESTs	4.14
206201_s_at	mesenchyme homeo box 2 (growth arrest-specific homeo box)	4.06
208019_at	zinc finger protein 157 (HZF22)	4.01
204991_s_at	neurofibromin 2 (bilateral acoustic neuroma)	3.97
207607_at	achaete-scute complex-like 2 (Drosophila)	3.88
AFFX-r2-Ec-bioD-5_at	E. coli /GEN=bioD /DB_XREF=gb:J04423.1 /NOTE=SIF corresponding to nucleotides 5024-5244 of gb:J04423.1 /DEF=E.coli 7,8-diamino-pelargonic acid (bioA), biotin synthetase (bioB), 7-keto-8-amino-pelargonic acid synthetase (bioF), bioC protein, and dethiobiot	3.83
211315_s_at	calcium channel, voltage-dependent, alpha 1G subunit	3.78
205953_at	leucine-rich repeats and immunoglobulin-like domains 2	3.75
207781_s_at	zinc finger protein 6 (CMPX1)	3.74

216068_at	Sodium- and chloride-activated ATP-sensitive potassium channel	3.69
214899_at	hypothetical BC331191_1	3.59
208212_s_at	anaplastic lymphoma kinase (Ki-1)	3.58

Table 2. Genes Down-regulated by LGD1069 in MDA-MB-231

probe set	gene	fold change
215117_at	recombination activating gene 2	-60.45
217535_at	Consensus includes gb:AV720514 /FEA=EST /DB_XREF=gi:10817666 /DB_XREF=est:AV720514 /CLONE=GLCGSB09 /UG=Hs.282721 ESTs, Weakly similar to ALU7_HUMAN ALU SUBFAMILY SQ SEQUENCE CONTAMINATION WARNING ENTRY H.sapiens	-16.22
201691_s_at	tumor protein D52	-16.09
207674_at	Fc fragment of IgA, receptor for	-6.54
215172_at	DKFZP566K0524 protein	-5.85
218541_s_at	chromosome 8 open reading frame 4	-5.79
215350_at	spectrin repeat containing, nuclear envelope 1	-5.69
AFFX-HUMRGE/M10098_5_at	H. sapiens /GEN=18S rRNA /DB_XREF=gb:M10098.1 /NOTE=SIF corresponding to nucleotides 115-595 of gb:M10098.1 /DEF=Human 18S rRNA gene, complete.	-5.59
213652_at	Proprotein convertase subtilisin/kexin type 5	-5.57
216050_at	Transcribed locus, moderately similar to NP_803425.1 DNA segment, Chr 19, Brigham & Women's Genetics 1357 expressed [Mus musculus]	-5.43
222342_at	Consensus includes gb:AW979196 /FEA=EST /DB_XREF=gi:8170484 /DB_XREF=est:EST391306 /UG=Hs.292713 ESTs, Moderately similar to ALU1_HUMAN ALU SUBFAMILY J SEQUENCE CONTAMINATION WARNING ENTRY H.sapiens	-5.41
205638_at	brain-specific angiogenesis inhibitor 3	-5.04
217464_at	Consensus includes gb:L48784 /DEF=050 Homo sapiens cDNA /FEA=mRNA /DB_XREF=gi:1066715 /UG=Hs.182426 ribosomal protein S2	-4.97
205848_at	growth arrest-specific 2	-4.86
206588_at	deleted in azoospermia-like	-4.75
213826_s_at	Consensus includes gb:AA292281 /FEA=EST /DB_XREF=gi:1940261 /DB_XREF=est:zt51b03.s1 /CLONE=IMAGE:725837 /UG=Hs.181307 H3 histone, family 3A	-4.74
220432_s_at	cytochrome P450, family 39, subfamily A, polypeptide 1	-4.48
209227_at	tumor suppressor candidate 3	-4.41
211712_s_at	annexin A9 /// annexin A9	-4.31
AFFX-HUMRGE/M10098_M_at	H. sapiens /GEN=18S rRNA /DB_XREF=gb:M10098.1 /NOTE=SIF corresponding to nucleotides 688-1219 of gb:M10098.1 /DEF=Human 18S rRNA gene, complete.	-4.28
AFFX-HUMRGE/M10098_3_at	signal recognition particle 68kDa	-4.2
202648_at	gb:BC000023.1 /DB_XREF=gi:12652562 /FEA=FLmRNA /CNT=966 /TID=Hs.298262.0 /TIER=ConsEnd /STK=0 /UG=Hs.298262 /LL=6223 /UG_GENE=RPS19 /DEF=Homo sapiens, ribosomal protein S19, clone MGC:1630, mRNA, complete cds. /PROD=ribosomal protein S19 /FL=gb:M81757.1 g	-4.15
207815_at	platelet factor 4 variant 1	-4.15
205363_at	butyrobetaine (gamma), 2-oxoglutarate dioxygenase (gamma-butyrobetaine hydroxylase) 1	-4.14
213856_at	CD47 antigen (Rh-related antigen, integrin-associated signal	-4.11

	transducer)	
216087_at	MRNA full length insert cDNA clone EUROIMAGE 117929	-4.11
211264_at	glutamate decarboxylase 2 (pancreatic islets and brain, 65kDa)	-4.03
220771_at	melanoma antigen	-3.83
220474_at	solute carrier family 25 (mitochondrial oxodicarboxylate carrier), member 21	-3.81
220281_at	solute carrier family 12 (sodium/potassium/chloride transporters), member 1	-3.8
217524_x_at	Consensus includes gb:AA018923 /FEA=EST /DB_XREF=gi:1482314 /DB_XREF=est:ze58d03.s1 /CLONE=IMAGE:363173 /UG=Hs.261204 ESTs	-3.72
211776_s_at	erythrocyte membrane protein band 4.1-like 3 /// erythrocyte membrane protein band 4.1-like 3	-3.69
212681_at	erythrocyte membrane protein band 4.1-like 3	-3.69
217333_at	Consensus includes gb:AL031903 /DEF=Human DNA sequence from clone 1032F13 on chromosome Xq25-26.3. Contains a pseudogene similar to Keratin 18 (KRT18, Cytokeratin 18) and ESTs /FEA=CDS /DB_XREF=gi:3766260 /UG=Hs.247763 Human DNA sequence from clone 1032F1	-3.69
210721_s_at	p21(CDKN1A)-activated kinase 7	-3.63
210327_s_at	alanine-glyoxylate aminotransferase (oxalosis I; hyperoxaluria I; glycolicaciduria; serine-pyruvate aminotransferase)	-3.57
206265_s_at	glycosylphosphatidylinositol specific phospholipase D1	-3.54
205847_at	protease, serine, 22	-3.52
202901_x_at	cathepsin S	-3.42
204681_s_at	Rap guanine nucleotide exchange factor (GEF) 5	-3.35
222227_at	Zinc finger protein 236	-3.35
207465_at	PRO0628 protein	-3.34

Table 3. Genes Up-regulated by LG100268 in MDA-MB-231

probe set	gene	fold change
219948_x_at	hypothetical protein FLJ21934	88.95
207750_at	gb:NM_018510.1 /DEF=Homo sapiens hypothetical protein PRO1866 (PRO1866), mRNA. /FEA=mRNA /GEN=PRO1866 /PROD=hypothetical protein PRO1866 /DB_XREF=gi:8924091 /UG=Hs.283031 hypothetical protein PRO1866 /FL=gb:AF119858.1 gb:NM_018510.1	26.42
209672_s_at	hypothetical protein FLJ20323	14.63
215514_at	Consensus includes gb:AL080072.1 /DEF=Homo sapiens mRNA; cDNA DKFZp564M0616 (from clone DKFZp564M0616). /FEA=mRNA /DB_XREF=gi:5262482 /UG=Hs.21195 Homo sapiens mRNA; cDNA DKFZp564M0616 (from clone DKFZp564M0616)	9.11
215309_at	Transcribed locus, weakly similar to XP_092995.4 zinc finger protein 21 (KOX 14) [Homo sapiens]	8.12
214774_x_at	trinucleotide repeat containing 9	7.58
203603_s_at	zinc finger homeobox 1b	5.77
205386_s_at	Mdm2, transformed 3T3 cell double minute 2, p53 binding protein (mouse)	5.2
205419_at	Epstein-Barr virus induced gene 2 (lymphocyte-specific G protein-coupled receptor)	4.18
216978_x_at	Consensus includes gb:U50277.1 /DEF=Human breast cancer suppressor element Ishmael Upper CP1 mRNA, partial cds. /FEA=mRNA /PROD=suppressor element Ishmael Upper CP1 /DB_XREF=gi:1224126 /UG=Hs.121485 Human breast cancer suppressor element Ishmael Upper CP	3.93
220931_at	hypothetical protein MGC5590	3.81
219995_s_at	hypothetical protein FLJ13841	3.77
208076_at	histone 1, H4d	3.6
214255_at	ATPase, Class V, type 10A	3.55
207987_s_at	gonadotropin-releasing hormone 1 (luteinizing-releasing hormone)	3.52
205651_x_at	Rap guanine nucleotide exchange factor (GEF) 4	3.46
220401_at	hypothetical protein FLJ21369	3.39
207241_at	chromosome 4 open reading frame 6	3.35
215623_x_at	SMC4 structural maintenance of chromosomes 4-like 1 (yeast)	3.17
216119_s_at	chromosome 20 open reading frame 28	3.13
217194_at	Consensus includes gb:AB007970.1 /DEF=Homo sapiens mRNA, chromosome 1 specific transcript KIAA0501. /FEA=mRNA /DB_XREF=gi:3413945 /UG=Hs.223020 Homo sapiens mRNA, chromosome 1 specific transcript KIAA0501	3.1
206381_at	sodium channel, voltage-gated, type II, alpha 2	3.09
212182_at	nudix (nucleoside diphosphate linked moiety X)-type motif 4	2.98
215112_x_at	MCF.2 cell line derived transforming sequence-like 2	2.94
213747_at	Consensus includes gb:AA047234 /FEA=EST /DB_XREF=gi:1525134 /DB_XREF=est:zf50b09.s1 /CLONE=IMAGE:380345 /UG=Hs.223014 antizyme inhibitor	2.84
221683_s_at	centrosome protein cep290	2.8
211611_s_at	tenascin XB /// tenascin XB /// cAMP responsive element binding protein-like 1 /// cAMP responsive element binding protein-like 1	2.74
205421_at	solute carrier family 22 (extraneuronal monoamine transporter), member 3	2.66
213764_s_at	microfibrillar associated protein 5	2.62
217505_at	hypothetical protein MGC22679	2.61

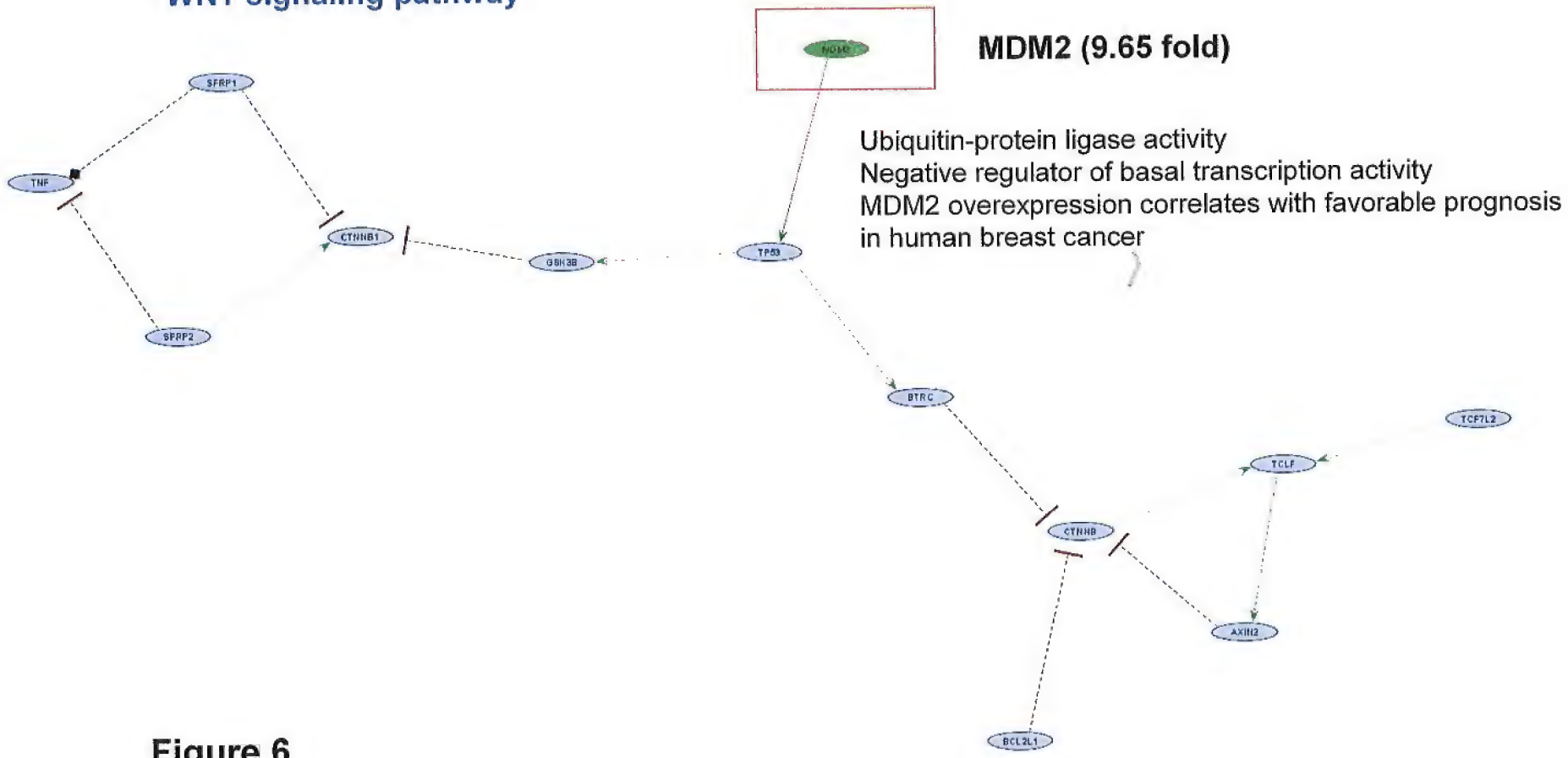
222320_at	Consensus includes gb:AW970584 /FEA=EST /DB_XREF=gi:8160429 /DB_XREF=est:EST382665 /UG=Hs.291033 ESTs	2.61
216466_at	Neuron navigator 3	2.59
AFFX-r2-Ec-bioB-M_at	E. coli /GEN=bioB /DB_XREF=gb:J04423.1 /NOTE=SIF corresponding to nucleotides 2393-2682 of gb:J04423.1 /DEF=E.coli 7,8-diamino-pelargonic acid (bioA), biotin synthetase (bioB), 7-keto-8-amino-pelargonic acid synthetase (bioF), bioC protein, and dethiobiot	2.55
216775_at	ubiquitin specific protease 53	2.54
206201_s_at	mesenchyme homeo box 2 (growth arrest-specific homeo box)	2.53
AFFX-BioDn-5_at	E. coli /GEN=bioD /DB_XREF=gb:J04423.1 /NOTE=SIF corresponding to nucleotides 4980-5256 of gb:J04423.1, not 100% identical /DEF=E.coli 7,8-diamino-pelargonic acid (bioA), biotin synthetase (bioB), 7-keto-8-amino-pelargonic acid synthetase (bioF), bioC pro	2.48
216894_x_at	cyclin-dependent kinase inhibitor 1C (p57, Kip2)	2.46
208019_at	zinc finger protein 157 (HZF22)	2.45
215803_at	hypothetical protein FLJ10178	2.44
222320_at	CDNA: FLJ23194 fis, clone REC00490	2.44

Table 4. Genes Down-regulated by LG100268 in MDA-MB-231

probe set	gene	fold change
217237_at	Zinc finger protein 423	-78.6
215014_at	Consensus includes gb:AL512727.1 /DEF=Homo sapiens mRNA; cDNA DKFZp547P042 (from clone DKFZp547P042). /FEA=mRNA /DB_XREF=gi:12224870 /UG=Hs.232127 Homo sapiens mRNA; cDNA DKFZp547P042 (from clone DKFZp547P042)	-17.74
213753_x_at	eukaryotic translation initiation factor 5A	-7.65
212382_at	Transcription factor 4	-5.74
AFFX-HUMRGE/M10098_5_at	H. sapiens /GEN=18S rRNA /DB_XREF=gb:M10098.1 /NOTE=SIF corresponding to nucleotides 115-595 of gb:M10098.1 /DEF=Human 18S rRNA gene, complete.	-5.58
211712_s_at	annexin A9 /// annexin A9	-5.49
209227_at	tumor suppressor candidate 3	-5.11
216917_s_at	synaptonemal complex protein 1	-4.82
AFFX-HUMRGE/M10098_M_at	H. sapiens /GEN=18S rRNA /DB_XREF=gb:M10098.1 /NOTE=SIF corresponding to nucleotides 688-1219 of gb:M10098.1 /DEF=Human 18S rRNA gene, complete.	-4.31
210697_at	zinc finger protein 257	-4.11
215013_s_at	ubiquitin specific protease 34	-3.97
209657_s_at	heat shock transcription factor 2	-3.96
221009_s_at	angiopoietin-like 4	-3.9
205612_at	multimerin 1	-3.79
207613_s_at	calcium/calmodulin-dependent protein kinase (CaM kinase) II alpha	-3.55
37232_at	KIAA0586	-3.38
AFFX-HUMRGE/M10098_3_at	signal recognition particle 68kDa	-3.37
204422_s_at	fibroblast growth factor 2 (basic)	-3.33
220638_s_at	Cas-Br-M (murine) ecotropic retroviral transforming sequence c	-3.32
208098_at	olfactory receptor, family 12, subfamily D, member 3 /// olfactory receptor, family 12, subfamily D, member 3 /// olfactory receptor, family 5, subfamily V, member 1 /// olfactory receptor, family 5, subfamily V, member 1	-3.29
213826_s_at	Consensus includes gb:AA292281 /FEA=EST /DB_XREF=gi:1940261 /DB_XREF=est:zt51b03.s1 /CLONE=IMAGE:725837 /UG=Hs.181307 H3 histone, family 3A	-3.25
208453_s_at	X-prolyl aminopeptidase (aminopeptidase P) 1, soluble	-3.2
207485_x_at	butyrophilin, subfamily 3, member A1	-3.18
211032_at	COBL-like 1 /// COBL-like 1	-3.11
220619_at	chromodomain helicase DNA binding protein 7	-3.04
209318_x_at	pleiomorphic adenoma gene-like 1	-3
201547_at	Jumonji, AT rich interactive domain 1B (RBP2-like)	-2.99
206996_x_at	calcium channel, voltage-dependent, beta 1 subunit	-2.98
220114_s_at	stabilin 2	-2.95
216709_at	Hypothetical gene supported by BC013370; BC034583	-2.93
203555_at	protein tyrosine phosphatase, non-receptor type 18 (brain-derived)	-2.92
213267_at	KIAA1117	-2.91
201122_x_at	eukaryotic translation initiation factor 5A	-2.89
213495_s_at	gb:AW166067 /DB_XREF=gi:6397592 /DB_XREF=xf44g10.x1 /CLONE=IMAGE:2620962 /FEA=EST /CNT=75 /TID=Hs.98614.2	-2.89

	/TIER=Stack /STK=51 /UG=Hs.98614 /LL=6238 /UG_GENE=RRBP1 /UG_TITLE=ribosome binding protein 1 (dog 180kD homolog)	
220301_at	chromosome 18 open reading frame 14	-2.88
214837_at	albumin	-2.85
209700_x_at	phosphodiesterase 4D interacting protein (myomegalin)	-2.84
216805_at	Transcribed locus, moderately similar to XP_375099.1 hypothetical protein LOC283585 [Homo sapiens]	-2.84
221671_x_at	immunoglobulin kappa constant	-2.79
214001_x_at	gb:AW302047 /DB_XREF=gi:6711724 /DB_XREF=xr52f08.x1 /CLONE=IMAGE:2763783 /FEA=EST /CNT=24 /TID=Hs.76230.2 /TIER=Stack /STK=20 /UG=Hs.76230 /LL=6204 /UG_GENE=RPS10 /UG_TITLE=ribosomal protein S10	-2.72
210047_at	solute carrier family 11 (proton-coupled divalent metal ion transporters), member 2	-2.69
208367_x_at	cytochrome P450, family 3, subfamily A, polypeptide 4	-2.66
219252_s_at	family with sequence similarity 51, member A1	-2.65
205827_at	cholecystokinin	-2.63

LGD1069-induced gene
; Apoptosis
- WNT signaling pathway



MDM2 (9.65 fold)

Ubiquitin-protein ligase activity
Negative regulator of basal transcription activity
MDM2 overexpression correlates with favorable prognosis in human breast cancer

Figure 6

LGD1069-induced gene ; Cell adhesion - IL1 signaling pathway

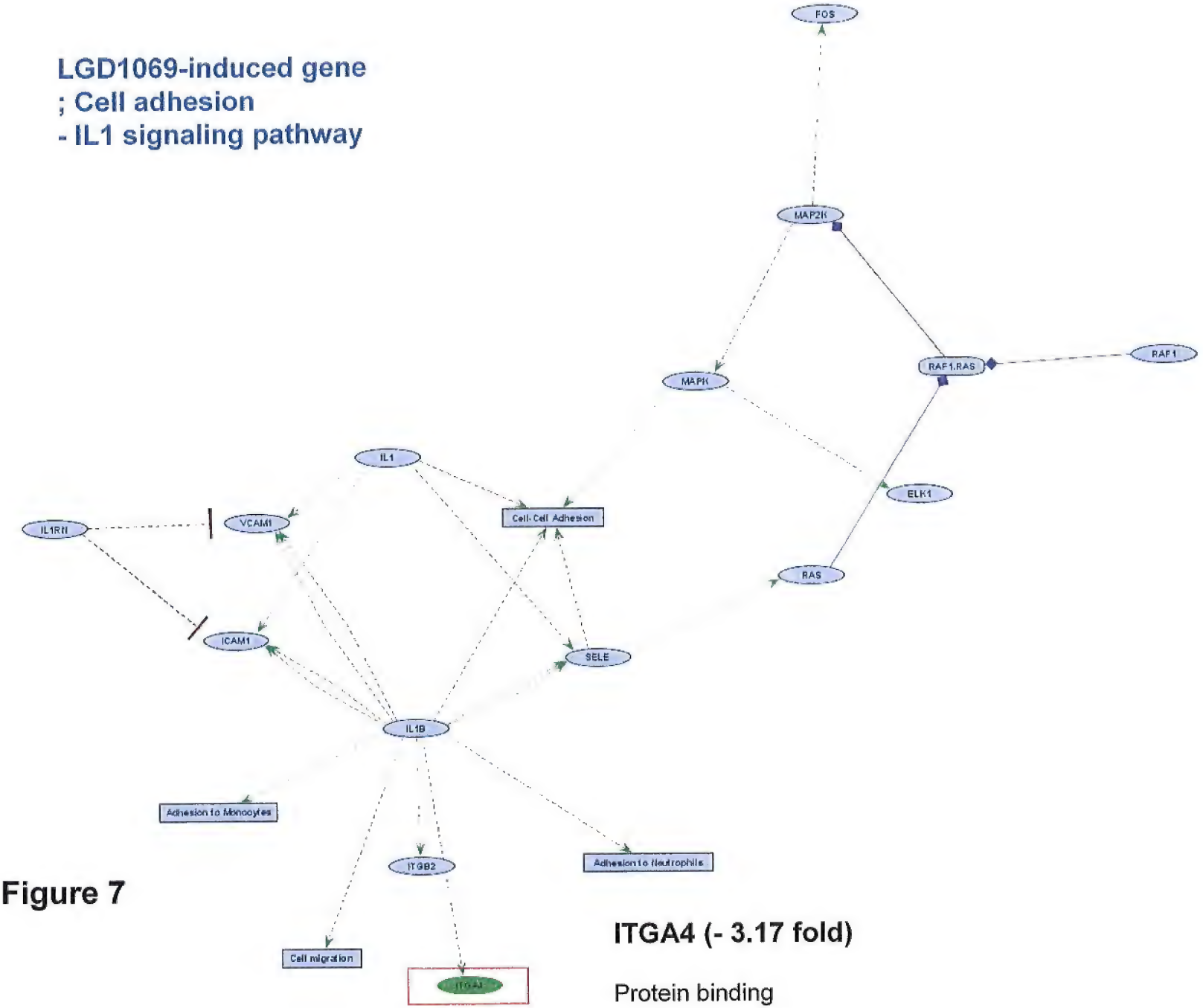


Figure 7

ITGA4 (- 3.17 fold)

Protein binding

LGD1069-induced gene
; protein family
- transcription factors

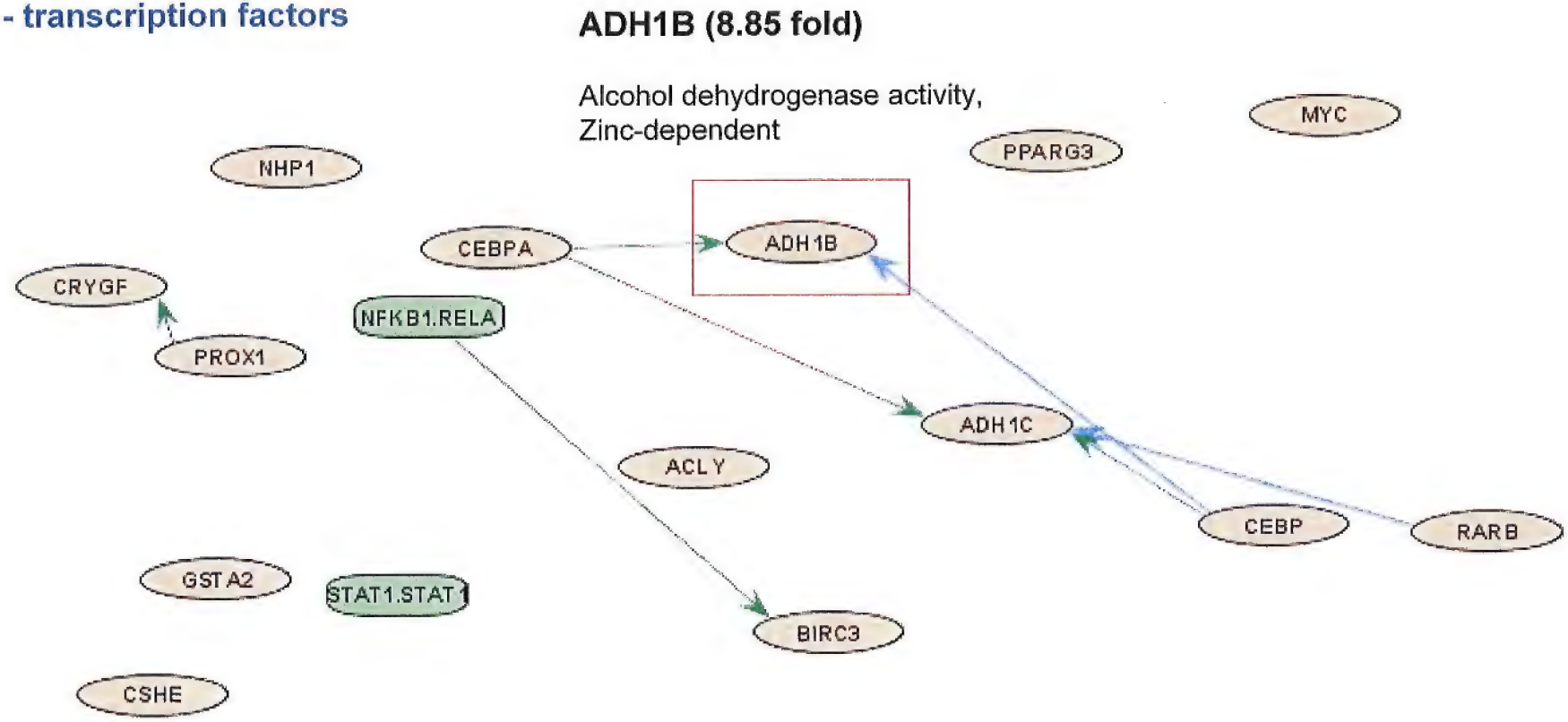
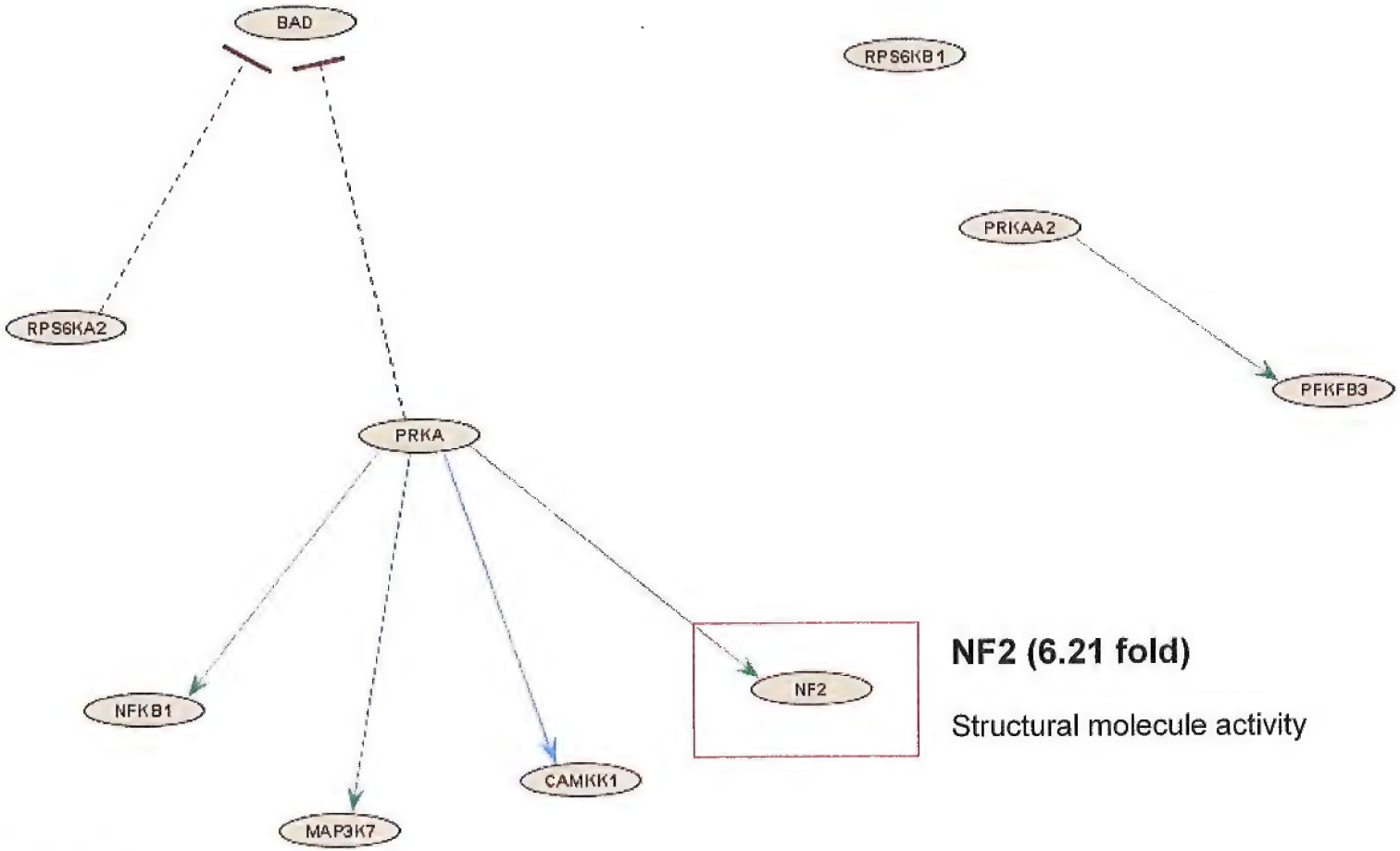


Figure 8

LGD1069-induced gene ; Protein family - kinase



NF2 (6.21 fold)

Structural molecule activity

Figure 9

LGD1069-induced gene
; Others
- WNT signaling pathway

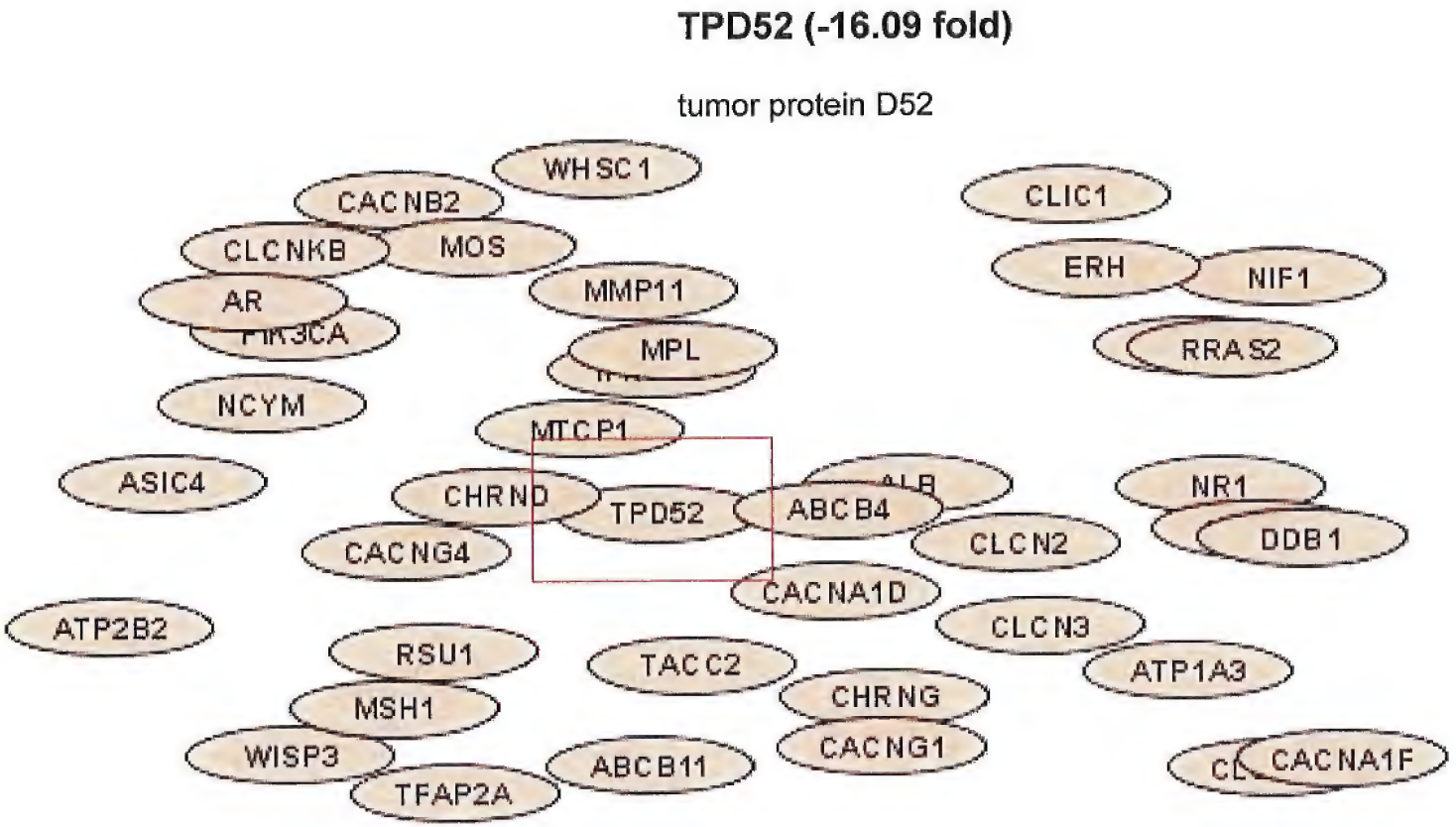
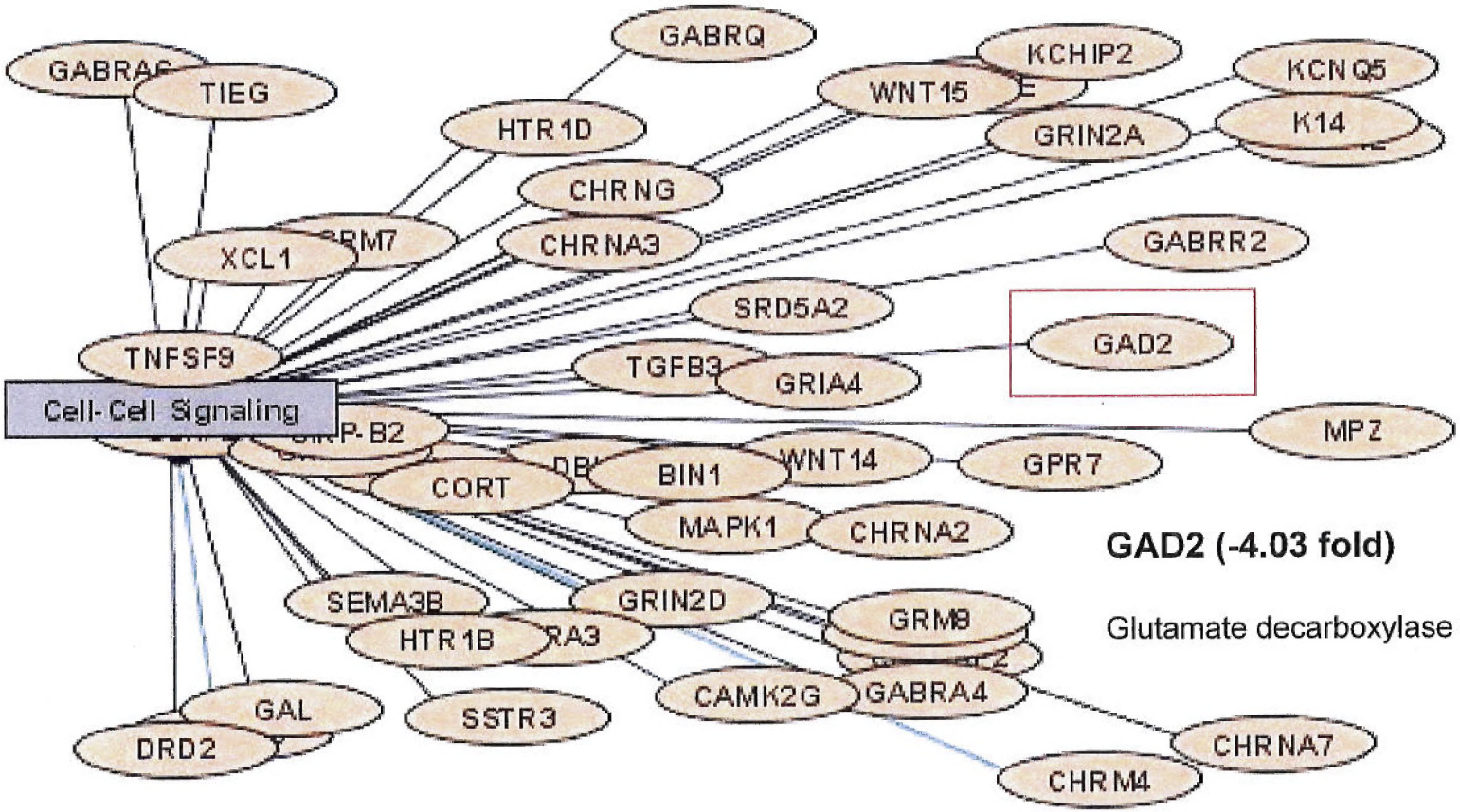


Figure 10

LGD1069-induced gene
; Others
- Cell-to-Cell signaling pathway

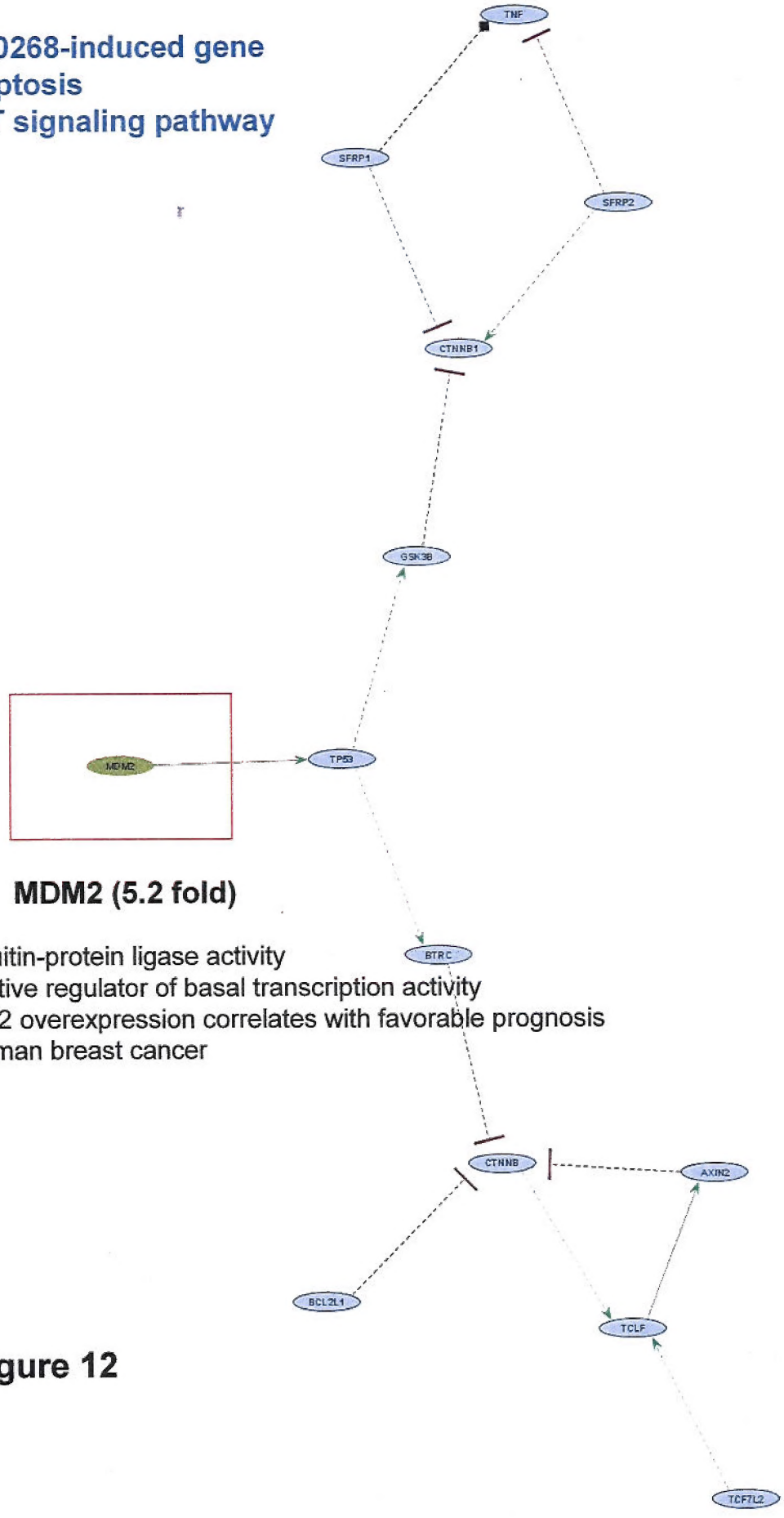


GAD2 (-4.03 fold)

Glutamate decarboxylase activity

Figure 11

LG100268-induced gene ; Apoptosis - WNT signaling pathway

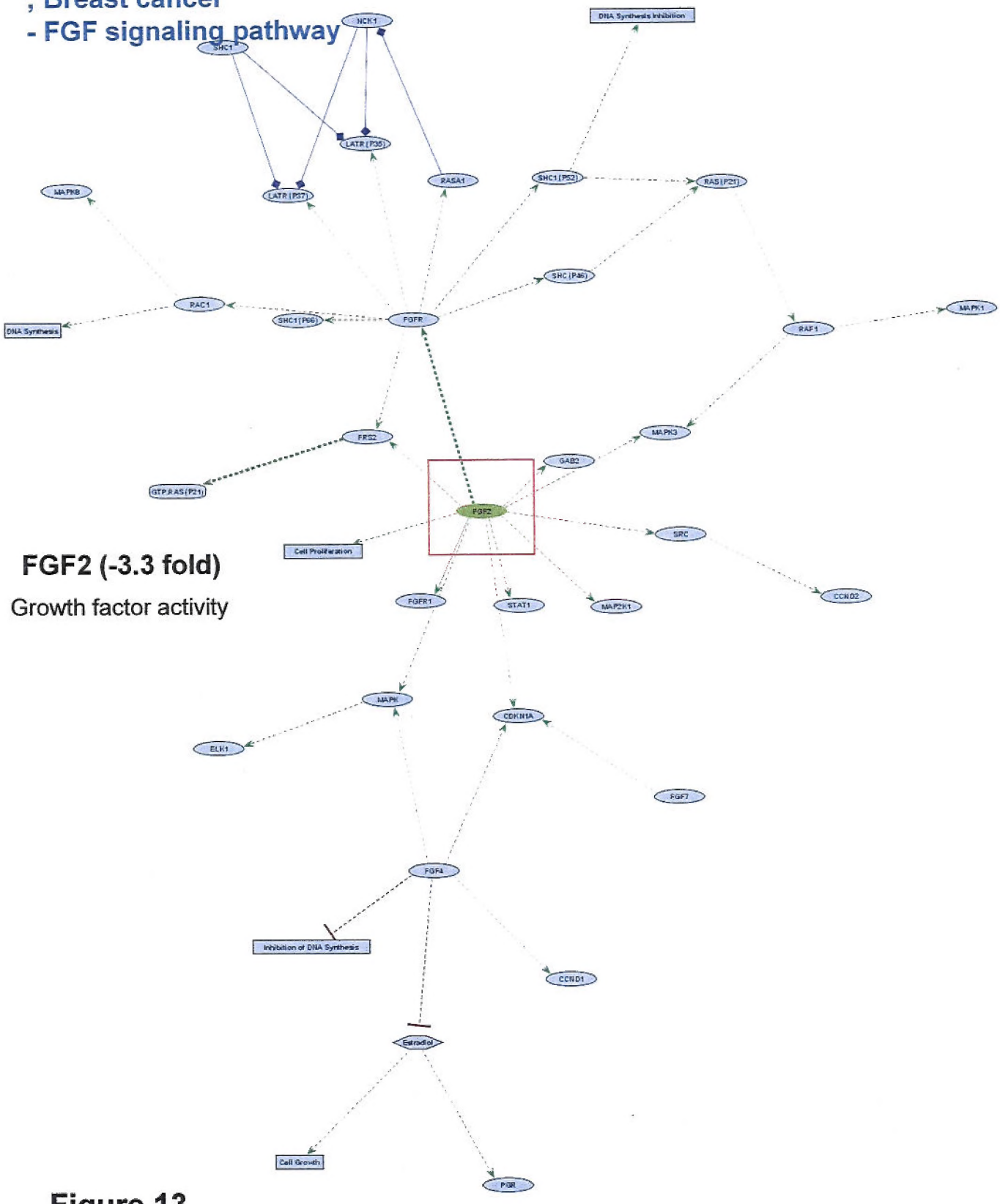


MDM2 (5.2 fold)

Ubiquitin-protein ligase activity
Negative regulator of basal transcription activity
MDM2 overexpression correlates with favorable prognosis in human breast cancer

Figure 12

LG100268-induced gene ; Breast cancer - FGF signaling pathway

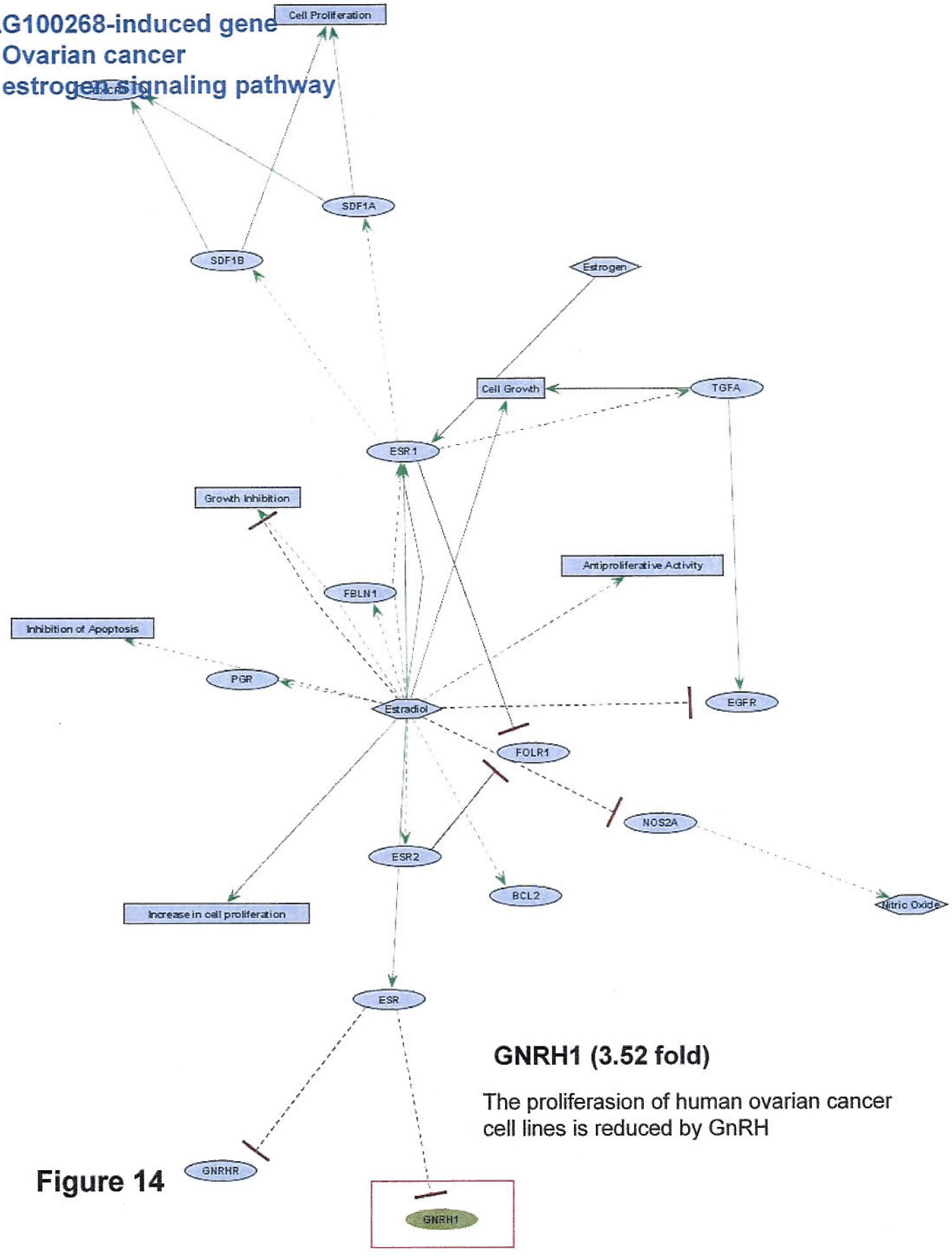


FGF2 (-3.3 fold)

Growth factor activity

Figure 13

LG100268-induced gene ; Ovarian cancer - estrogen signaling pathway



GNRH1 (3.52 fold)

The proliferasion of human ovarian cancer cell lines is reduced by GnRH

Figure 14